HAWAII AGRICULTURAL EXPERIMENT STATION
HONOLULU, HAWAII

Under the supervision of the
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CARBOHYDRATE METABOLISM AND ITS RELATION TO GROWTH IN THE EDIBLE CANNA

BY

J. C. RIPPERTON, Chemist

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By J. C. Ripperton, *Chemist*

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The potential importance of edible canna (*Canna edulis*) as a commercial source of starch has led to various experiments both at the central station in Honolulu and at Waimea, Hawaii. Although the plant is well known botanically, and has been grown in Queensland, Australia, for many years for starch, the available literature gives no information regarding its production on a field scale. Field methods of study devised by the station have given considerable data on the growth of the plant. Laboratory studies of the carbohydrate metabolism of the plant have been made to learn the significance of the field results.

HABIT OF GROWTH OF THE EDIBLE CANNA

The edible canna belongs to the same genus as the familiar flowering variety, and resembles it both in appearance and manner of growth. Starting with the rootstock and the original stem, growth takes place through the development of axillary buds growing from the nodes of the rootstock. Growth within the hill is rapid when conditions are favorable. Development of the bud follows closely development of the parent, so that in a vigorous hill as many as 10 plants,[1] representing three to five generations (fig. 1), may be growing simultaneously. As the plant reaches maturity, first the leaves and then the stem shrivel, and the rootstock becomes dormant, while new growth continues through the developing buds.

[1] The term "plant" is used to denote a single rootstock and its attached stem.

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The storage organ or lower part of the plant is essentially a rootstock, or part of the stem in which starch is stored, and morphologically it is an integral part of the stem. In some of the types of slender rootstock it is difficult to determine just where the rootstock ends and the stem proper begins.

Under normal conditions the new rootstocks attain very considerable size with energy supplied by the parent plant, their own stems remaining entirely undeveloped. (Figs. 1, 3, 4, 5, 6, and 7) When vigorous growth of the meristem decreases, the internodes at the apex shorten very rapidly, and the stem develops. Under optimum conditions, the rootstock continues to increase in size for some time, as is shown by the large, fresh cracks on its surface. Increase in size, however, may be the result of cell elongation rather than of cell growth. The potency of the parent stem is strikingly shown by the fact that when all the mature top is removed the hill produces extremely stunted rootstocks. (Fig. 8.)

FIG. 1.—A hill of canna, 6 months old, at its maximum growing stage. The stems of the first three generations have bloomed and are functioning at their maximum in producing sugars for the storage of starch and for the production of new growth. Note the large, vigorous new rootstocks

The first generation rootstocks are always small and cylindrical in type; the second, and sometimes the third generation, termed the intermediate type, have a definite tapering shape. They grow below the surface of the ground, starting with a small attachment to the parent, and increase in diameter to a definite bulge approaching the apex. The third and fourth generations, under ordinary cultural methods, grow at or near the surface of the ground, starting with a large attachment to the parent. They are oval to spherical and largely develop above the surface of the ground. (Fig. 3.) Subsequent generations are of thissame general surface type.

The characteristic shapes (fig. 9) may be attributed largely to the number of antecedent stems upon which the developing rootstock has to draw. The first generation has none The second generation, which closely follows the first, receives little plant food from the par-

ent at first, but increases in diameter, assuming a tapering shape, as the quantity of food received increases. The surface type, which has two or more stems on which to draw, starts with a large attachment to the parent and develops an oval shape.

Buds are produced in the axils of the scales of the rootstock. They are sometimes produced in profusion on the cylindrical type of rootstock, but most of them remain dormant. Ordinarily three buds develop on rootstocks of the intermediate type, being attached at intervals from near the base to the apical part of the mature rootstock. Usually only two vigorous buds grow on rootstocks of the surface or oval type, and they are attached near the apex. A number of other buds, termed "top buds," which are very small and dormant, grow at the very short nodes near the extreme apex of the rootstock. (Fig. 10.) Normally, these do not develop, although some of them may grow when the rootstock is used for seed.

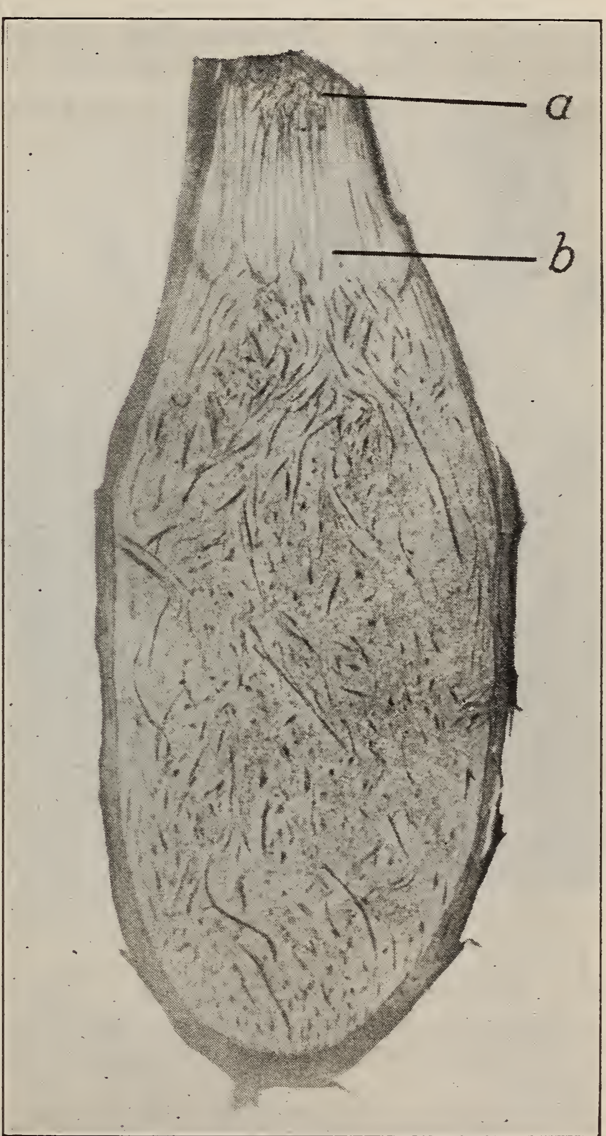

FIG. 2.—Longitudinal section of an intermediate type of rootstock. A is the first node of the stem; B is the apex of the rootstock. The characteristic parallelism of the vascular bundles in the stem differentiates it from the rootstock proper

The vigor of the developing bud seems to depend upon the age of the parent rootstock. A bud has maximum vigor if it starts to grow while the parent is young and rapidly developing in the region of bud attachment. Growth is stunted, however, and the size of the rootstock ultimately greatly decreases, if bud development is retarded until the parent is old (fig. 8); hence, the edible canna requires uniform growing conditions to make the best growth.

Fields of canna failing to send out much new growth under adverse conditions may produce new stems in profusion with the return of

favorable conditions, but the newly formed rootstocks are very much undersize. Grouping the hill according to generations shows that the new growth is the result of bud development on all the generations. The stunted growth is termed "secondary" in contrast with the vigorous "primary" growth starting while the parent rootstock is young. (Figs. 11 and 12.)

A number of more or less distinct stages occur during the growth of a hill of canna. The first stage covers the establishment of the plant, which often requires as long as three months, during which the first two generations develop. The rootstocks lie almost entirely beneath the surface of the ground and usually are small and comparatively low in starch content. The second stage is one of rapid development of new rootstocks (fig. 4), the third, fourth, and fifth generations developing in quick succession. These are of the oval-surface type and usually are large. After these generations have developed the new growth generally declines in vigor, and the rootstocks decrease in size. The original stem dies, and the stems of subsequent generations become less vigorous. With the decline in vigor of the primary growth, the secondary growth begins, producing smaller rootstocks. The new growth thenceforth continues to produce stems of diminishing vigor and rootstocks decreasing in size until they ultimately become undesirable for starch making.

FIG. 3.—A close-up view of the base of a hill 4 months old. As is characteristic of the canna plant, the newest growth is mostly on top of the ground

These stages of growth are not so apparent at Waimea, Hawaii, where the plant seems to be perfectly adapted to both soil and climatic conditions. One or more "lines" in the hill may continue to produce large primary growth for an indefinite number of generations notwithstanding the gradual decrease in the average weight of the new rootstocks in the later stages of growth.

METHODS OF INVESTIGATION

FIELD METHODS

Two general methods were devised for continuous study of the growth of edible canna.

In the first method, the different generations of the rootstock were studied, tracing the progressive growth of the hill from the original seed rootstock. The hill was carefully dug and the individual plants were separated and grouped according to successive generations, beginning with the plant developing from the original seed rootstock as the first generation. Notes were then made of the number, kind, and size of the rootstocks of each generation, the

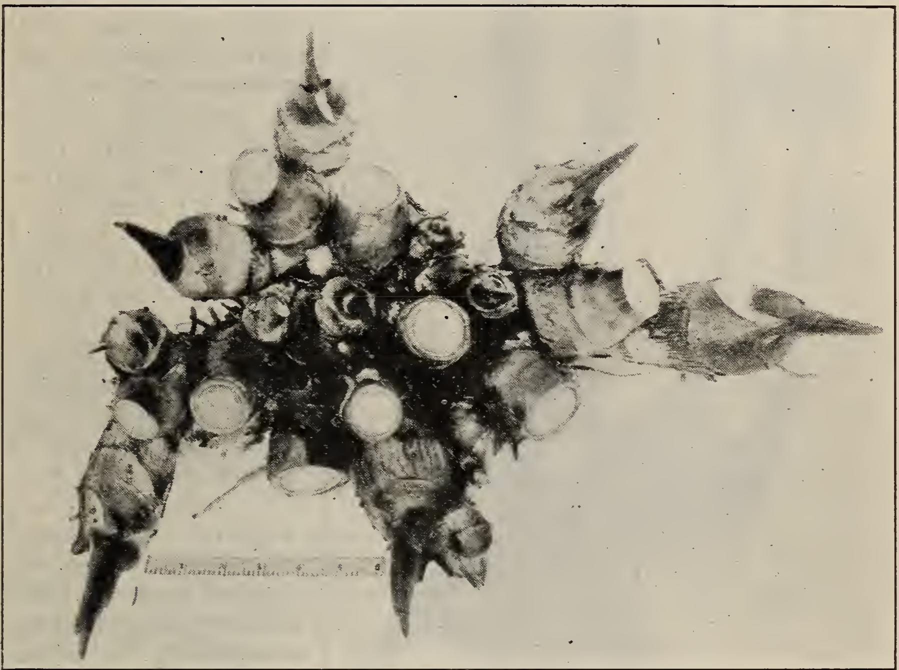

FIG. 4.—Top view of a hill of canna during its optimum growing stage (4 months old). The seven large spike rootstocks, all of the fourth generation, were supported by nine vigorous stems. Note the profusion of smaller secondary "spikes" developing in the center of the hill

stage of maturity of stems, and the amount of both primary and secondary growth.

In the second method, the classification of rootstocks was studied, the lot from each hill being grouped as dormant, mature, and immature.

The procedure followed was to dig the hill of canna and group the different plants in the hill according to the stage of maturity of their stems. Group 1, dormant stage, comprised plants on which the leaves had died and the stem had partly or wholly shriveled. This group appears when the hill is 8 to 12 months old. Group 2, mature stage, comprised plants whose new growth within the stem had ceased, and, in case of the older members, the lower leaves had shriveled. Group 3, immature stage, comprised plants the stems of which had not attained their maximum growth. Group 3 was subdivided into two lots (*a*) plants with stems developing, comprising

the older members of the group including developing stems with more than one leaf, and (*b*) plants with stem undeveloped, composed chiefly of "spike" rootstocks.

LABORATORY METHODS

Determination was made of the reducing and nonreducing sugars in the sap of the plant. Immediately after the plant was dug the parts to be analyzed were ground in a meat chopper and the juice was expressed, when necessary, by means of a screw press.[2] Usually the tough fibers of the plant clogged the meat chopper and produced a combined grinding and pressing effect. The resulting pulp was very dry and the expressed sap could be drawn off at the opposite end of the grinder thus omitting the use of the press. Twenty-five cubic centimeters of the sap was immediately pipetted out for determination of reducing and nonreducing sugars. Clarification of the sap by means of neutral lead acetate solution was not always successful. Certain plant substances, probably some of the gums, which failed to be precipitated by the lead acetate solution, were subsequently thrown down by the Fehling solution, during the determination of reducing sugars as a flocculent precipitate.

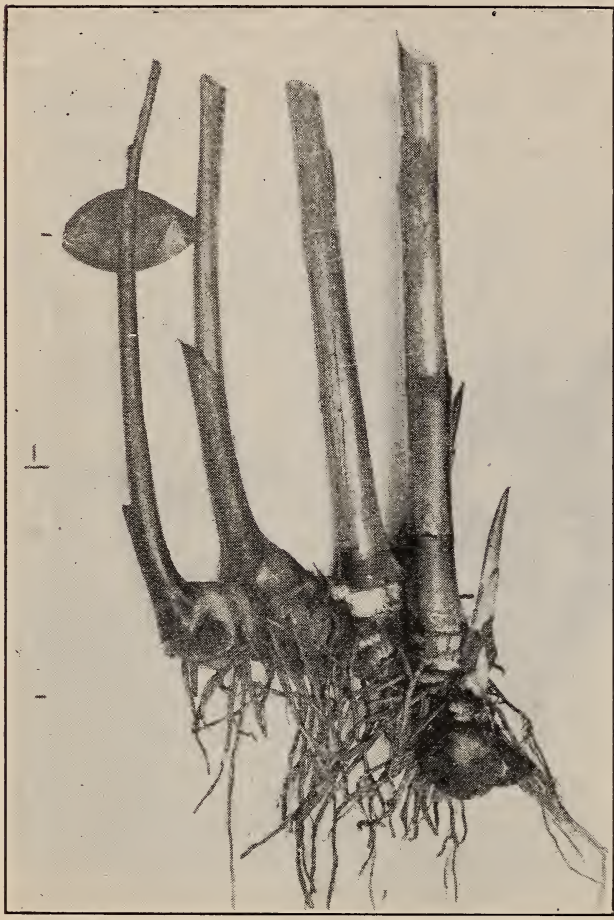

FIG. 5.—Four generations of rootstocks. Note the progressive change in shape and tendency of the rootstocks to grow out of the ground. Secondary growth is appearing on the first-generation rootstock

To eliminate this difficulty the following procedure was followed: Fifty cubic centimeters of 95 per cent ethyl alcohol was poured into 25 cubic centimeters of the freshly expressed sap stirred continuously. This produced a flocculent precipitate which was easily filtered out. Fifty cubic centimeters of the filtrate was pipetted[3] into a beaker

[2] This method is subject to criticism when the leaves are used because they contain invertase which may cause rapid inversion of the sucrose. This trouble is reduced to a minimum, however, when only stems and roots are used because of their very low invertase content. Evidently very little inversion took place since the hexose content in all the leaf samples was very low. (See Table 6.)

[3] Shrinkage of volume, of course, occurs upon the addition of alcohol to sap, which fact is taken into account when the percentage of sugars is computed.

and evaporated over a steam bath to about 15 cubic centimeters. Water was then added and the solution transferred to a flask graduated to 100 cubic centimeters and clarified with neutral lead-acetate solution. After this, the regular method was adhered to, the reducing sugars being determined by the method of Munson and Walker (*1, p. 78*).[4] Inversion was effected by allowing the solution to stand with hydrochloric acid for 12 hours at room temperature. In all cases the hexoses were calculated as invert sugar.

PRESERVATION OF SAP SAMPLES FOR ANALYSIS

Obviously, it was necessary to prevent changes in the sugars of sap samples collected in the field. In the first trial 25 cubic centimeters each of sap from rootstock and stem was treated with formalin (40 per cent formaldehyde), stored for three days, and analyzed. Table 1 shows the changes taking place in the sugars of the sap on standing with formalin as a preservative.

TABLE 1.—*Effect of formalin (40 per cent formaldehyde) on the sugars of samples of sap from the canna plant*

Test No.	Source of sap and treatment with formalin	Sucrose		Hexoses		Total sugars	
		Fresh sample	Stored 3 days	Fresh sample	Stored 3 days	Fresh sample	Stored 3 days
		Per cent	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*
1	Rootstock (4 drops)	0.95	0.86	0.09	0.21	1.04	1.07
	Rootstock (12 drops)	.95	.83	.09	.37	1.04	1.20
2	Do	1.01	.93	.15	.37	1.16	1.30
3	Stem (4 drops)	.42	.13	.39	.72	.81	.85
	Stem (12 drops)	.42	.19	.39	.75	.81	.94
4	Do	.27	.08	1.26	1.56	1.53	1.64

Formalin did not prevent inversion of sucrose, although there was no loss in total sugars when it was used; in fact, the total sugars slightly increased in the juices treated with formalin, the percentage of sugars increasing with the increased quantities of formalin used.

Ethyl alcohol was next tried as a preservative, 25 cubic centimeters of sap being pipetted into 50 cubic centimeters of 95 per cent alcohol that had been freshly prepared by distillation with sodium hydroxide. Two procedures were followed. In one the samples were filtered at once, and the clear alcoholic filtrate stored; in the other the unfiltered samples were stored. Table 2 gives the results of the tests.

TABLE 2.—*Effect of ethyl alcohol on the sugars of samples of sap from the canna plant*

Sample No.	Source of sap and treatment	Sucrose	Hexoses	Total sugars
		Per cent	*Per cent*	*Per cent*
1	Stem, fresh	0.34	1.45	1.79
2	Stem, not treated, stored 3 days	.02	.71	.73
3	Stem, stored in alcohol 3 days, then filtered	.28	1.46	1.74
4	Stem, alcoholic filtrate stored 3 days	.30	1.45	1.75
1	Rootstock, fresh	1.16	.23	1.39
2	Rootstock, not treated, stored 3 days	----------	.07	.07
3	Rootstock, stored in alcohol 3 days, then filtered	1.15	.25	1.40
4	Rootstock, alcoholic filtrate stored 3 days	1.14	.25	1.39

[4] Reference is made by number in italics to Literature cited, p. 34.

Ethyl alcohol, used in the proportion of 2 parts to 1 of sap, prevented changes in the sugars. The sugars largely disappeared in the untreated sap. Since, as the results show, it is not necessary to filter off the alcohol precipitate before storage, 50 cubic centimeters of alcohol was pipetted into 4-ounce glass-stoppered bottles in the laboratory for use in the field. Samples of the canna plant were collected and 25 cubic centimeters of the sap was, in each instance, expressed and pipetted into the containers in the field.

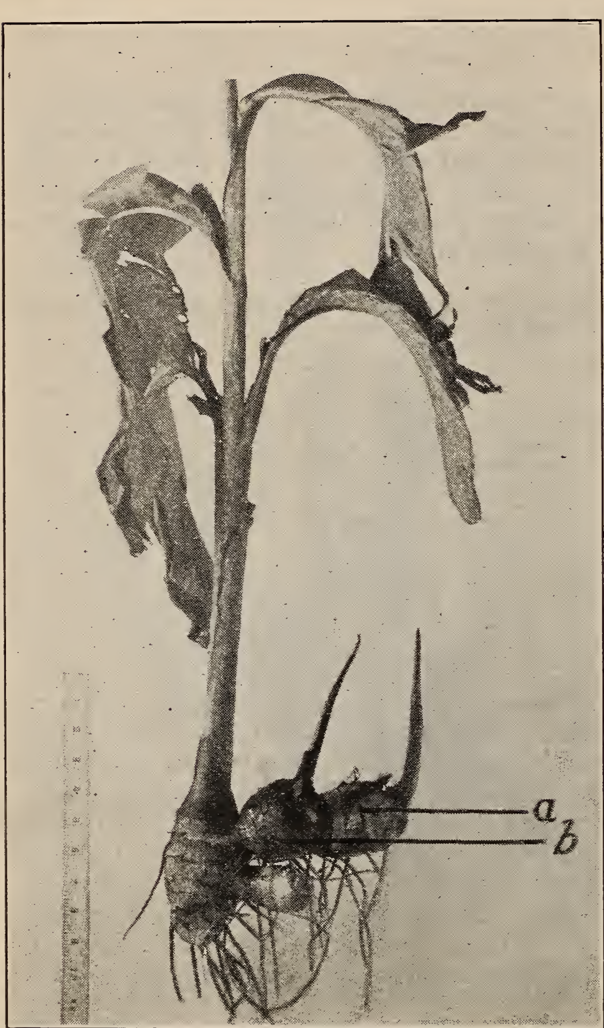

FIG. 6.—Two generations of "spike" rootstocks produced from a single stem. A, the second generation, is developing its stem. The meristem of B, the first generation, was killed. The upper part of the parent stem was removed

DETERMINATION OF STARCH

Starch was determined in the dry samples by the diastase method recommended by the Association of Official Agricultural Chemists (*1, p. 95*), the resultant sugars being determined by the method of Munson and Walker (*1, p. 78*).

The specific gravity of the rootstocks was determined by the Westphal balance, kerosene being used as the immersion medium. The use of water was not feasible because the difference in its density and that of the canna rootstock is too small. The latter sometimes has a specific gravity less than water. Kerosene, with a density of 0.815 at room temperature, gives a convenient difference in density, and has the additional advantage that it causes portions of the sap exuding from the cut surfaces of the rootstock to precipitate undissolved to the bottom of the container. The rootstock was suspended by means of a fine wire connected with a small screw driven into the rootstock. All roots, dead scales, and adhering soil, as well as the stem, were carefully removed from the rootstock before determination was made. The removal of the stem at the exact apex of the rootstock is important since the specific gravities of the stem and the apical and basal parts of the rootstock are

very different. The inclusion of a part of the stem or the removal of the apical part of the rootstock would thus introduce serious error into the determination of the specific gravity of the rootstock as a whole.

SPECIFIC GRAVITY AND ITS RELATION TO STARCH CONTENT OF ROOTSTOCK OF DIFFERENT AGES

The method used to determine specific gravity has been described. Table 3 shows the variations in specific gravity of canna rootstocks grouped according to stage of maturity, generation, and age of hill.

TABLE 3.—*Specific gravity of canna rootstocks of different ages*

Group	3 months old		5 months old		7½ months old		9 months old		22 months old	
	Generation	Specific gravity	Generation	Specific gravity	Generation	Specific gravity	Generation	Specific gravity	Generation[1]	Specific gravity
1							First	1.030		1.080
							Second	1.088		
2	First	1.034	First	1.065	First	1.073	Third	1.102		1.104
	Second	1.000	Second	1.032	Second	1.104				
					Third	1.089				
3a	Third	.977	Third	1.030	Fourth	1.054	Fourth	1.093		1.070
							Fifth	1.073		
							Sixth	1.086		
							Seventh	1.061		
3b	Fourth	.971	Fourth	.990	Fifth	1.032				

[1] Not genealogized. When the hill reaches this stage it is practically impossible to separate according to generations because of its size.

A number of variations are evident in Table 3. Each hill, considered as a whole, showed a gradual rise in specific gravity with advancing age. Starting with the 3-months-old hill having an average specific gravity of less than 1, the specific gravity continued to rise to the ninth month; thence to the twenty-second it varied slightly. In the early growth of the hill the first generation had the highest specific gravity, each succeeding generation showing a decreasing value. From seven and one-half months the second or third generation had the highest value; thence the value continued to decrease. The specific gravity of Group 2 was highest throughout; that of Groups 3a and 3b decreased, whereas that of Group 1 was variable, but always less than in Group 2. The average specific gravity of the canna rootstock is below that of the potato (*16*), and the range of variation is much greater, owing largely to the fact that young and immature rootstocks as well as mature ones are found in every hill of canna, regardless of its age.

To ascertain the relationship between the specific gravity, determined by the method outlined by Wiley (*16, pp. 369–371*), and the other constituents of the canna rootstock, a series of analyses was made of hills of different ages and from different localities. Table 4 shows the relationship of the specific gravity to the starch, dry matter, and solids other than starch in the canna rootstock.

TABLE 4.—*Relation of the specific gravity of rootstocks, as shown by stage of maturity, to their starch content and other constituents*

HILL A (12 MONTHS OLD), STATION FIELD 26A

Sample No.	Generation	Stage of maturity	Moisture	Dry matter	Starch in Green weight	Starch in Dry weight	Solids other than starch	Specific gravity
			Per cent	*Per cent*	*Per cent*	*Per cent*	*Per cent*	
1		Group 1	55.30	44.70	21.30	70.03	13.40	1.056
2		Old Group 2	70.72	29.28	22.53	76.93	6.75	1.106
3		Old Group 3a	73.91	26.09	19.92	76.36	6.17	1.094
4		Young Group 3a	82.70	17.30	11.12	64.30	6.18	1.033
5		Group 3b (spike)	84.58	15.42	8.96	58.11	6.46	1.012
HILL B (7½ MONTHS OLD), STATION FIELD 26B								
6	First	Old Group 2	70.15	29.85	17.49	58.58	12.36	1.073
7	Second	Medium Group 2	63.90	36.10	28.81	79.81	7.29	1.104
8	Third	Young Group 3a	68.22	31.78	22.83	71.83	8.95	1.089
9	Fourth	Medium Group 3a	79.46	20.54	15.18	73.89	5.36	1.054
10	Fifth	Young Group 3a	86.40	13.60	9.47	69.64	4.13	1.032
HILL C (3½ MONTHS OLD), STATION FIELD 32C								
11	First	Medium Group 2	80.52	19.48	11.51	59.08	7.97	1.034
12	Second	Young Group 2	85.52	14.48	7.75	53.54	6.73	1.000
13	Third	Medium Group 3a	87.42	12.58	7.20	57.20	5.58	.977
14	Fourth	Young Group 3a	90.42	9.58	4.59	47.91	4.99	.974
15	do	Group 3b (spike)	91.92	8.08	2.58	31.87	5.50	.971
HILL S (11 MONTHS OLD), WAIMEA FIELD S								
16	First	Group 1	65.23	34.77	27.42	78.85	7.35	1.097
17	Second	Old Group 2	65.25	34.75	27.92	80.35	6.83	1.115
18	Third	Medium Group 2	70.47	29.53	24.41	82.65	5.12	1.104
19	Fourth	Young Group 3a (old)	85.71	14.29	9.42	65.94	4.87	1.021
20	do	do	85.86	14.14	9.83	69.52	4.31	1.027
21	do	do	80.17	19.83	13.60	68.58	6.23	1.051
22	Fifth	Spike, dead	75.20	24.80	18.59	74.96	6.21	1.076
23		Young Group 3a (fresh)	90.68	9.32	4.51	48.37	4.81	.972
24		do	92.79	7.21	2.08	28.85	5.13	.986

A distinct correlation between specific gravity and starch and other constituents is apparent. Certain discrepancies exist, particularly with respect to the first generation rootstocks, which were characterized by unusually high solids other than starch. In the older hills the specific gravities of such rootstocks were less than of second generation rootstocks, and bore slight relation to the percentages of starch. As previously stated (p. 2), this kind of rootstock is materially different in manner of growth from the other rootstocks in the hill, and since only one or two grow in each hill, they may be disregraded in considering the hill as a whole. Although the solids other than starch did not vary widely, they showed a distinct tendency to follow the specific gravity curve. The relationship between these several constituents is evident, particularly in case of hill S (fig. 13),[5] in which the percentages dropped suddenly in the fourth generation, increased in the fifth, and dropped again in the sixth generation.

[5] The first generation of each hill is omitted from figs. 13 and 14.

If the specific gravity and starch content of the several hills are plotted as separate curves (fig. 14), each hill would seem to have what might be termed a characteristic "growth curve," the location and nature of which depends upon a number of factors, including age, rainfall, and rapidity of growth.

The curve of hill S, from Waimea rootstocks, covering a wide range of percentages and lying about midway of the curves of hills A and B, was adopted for field studies and mill control. Table 5, taken from the curve of hill S, shows the approximate starch content of rootstocks as ascertained by the specific gravity.

TABLE 5.—*The starch content of rootstocks as determined by specific gravity*

Specific gravity	Starch in rootstock	Specific gravity	Starch in rootstock	Specific gravity	Starch in rootstock
	Per cent		*Per cent*		*Per cent*
0.98	3.5	1.03	10.2	1.08	18.7
.99	4.7	1.04	11.7	1.09	20.9
1.00	6.0	1.05	13.3	1.10	23.3
1.01	7.4	1.06	15.0	1.11	26.1
1.02	8.8	1.07	16.8	1.12	30.3

Determination of the starch content of a rootstock by its specific gravity is admittedly only approximate. The only varying factors considered are starch and water; differences in structure or of air spaces within the rootstock are not taken into account. The latter, particularly, introduces appreciable error in the determination. The specific gravity of a canna rootstock may be increased from 1.07 to 1.12 and higher by placing the rootstock in water and evacuating. Notwithstanding these imperfections the method has much practical value in both field and factory. Obviously, its accuracy depends upon the number of analyses from which the table is constructed, and many additional analyses will have to be made before a thoroughly reliable table can be made.

CARBOHYDRATE METABOLISM

Variations in the sugars of the sap of the canna plant offer special advantages to the chemist in studying its methods of growth as a starch crop. The relationship between the sugars and the growth of the plant can be learned, as well as the various changes taking place in form and concentration of the sugars during translocation and ultimate storage as starch.

Study of the occurrence of sugars in the metabolism of the plant and their significance began with the discovery by Sachs (*12*) in 1862 of starch in the chlorophyll-granule and his conclusion that starch disappears from the leaf by conversion into sugar. Schimper (*13*) modified Sach's hypotheses by stating that glucose formation precedes starch formation in the leaf, and that starch is formed from glucose when its concentration exceeds a certain maximum, which varies in different plants.

Meyer (*10*) observed that the leaves of many plants contain no starch, and that such leaves usually have a high sugar concentration compared with those containing starch. Later, Brown and Morris (*2*), using more exact chemical methods, studied the nature of the

sugars and found that the reducing sugars, which up to that time had been considered as glucose, consist of both levulose and dextrose. The presence in the leaf of large quantities of sucrose and the nature of its fluctuations led to the conclusion that sucrose is the first sugar produced by photosynthesis, and that levulose and dextrose are the products of hydrolysis of the sucrose.

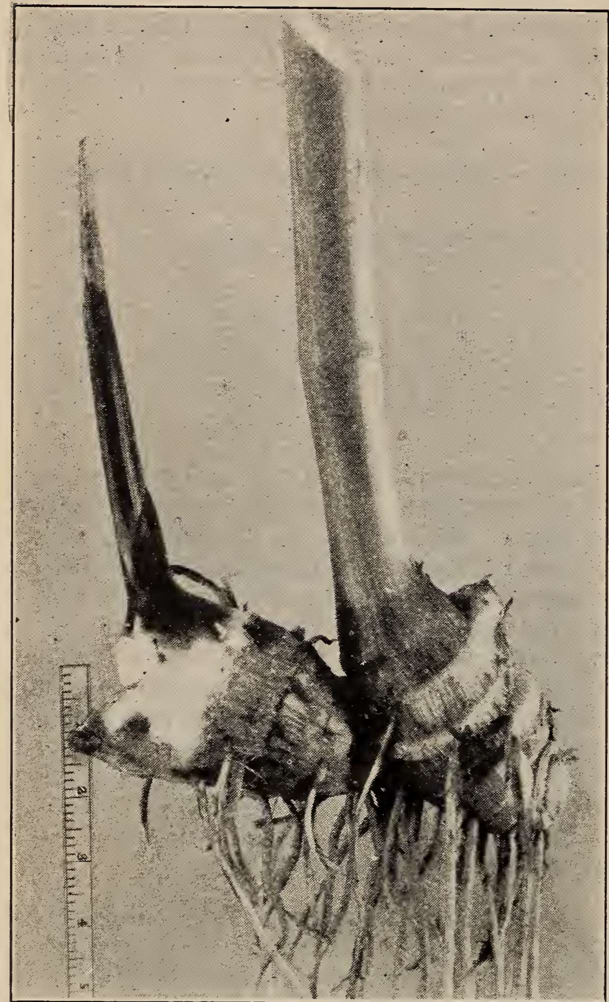

FIG. 7.—Different stages in the development of the bud. The stem of the large offspring is developing, resulting in a sharp decrease in cell growth at the apex of the rootstock. This has led to vigorous development of its bud. The bud on the parent rootstock has failed to develop, and probably would eventually result in a small secondary rootstock

Davis, Daish, and Sawyer (*6*), in studying the carbohydrates of the mangel leaf, and Davis and Sawyer (*5*), in studying the potato plant, found that, whereas in the leaf sucrose predominates, the hexoses are in excess of the sucrose in the midribs and stems. From this they concluded that sucrose is the "primary sugar formed in the mesophyll of the leaf under the influence of the chlorophyll," and that "it is transformed into hexoses for the purpose of translocation" (*6, p. 314*). Strakosch (*14*) took the opposite view. Using microchemical methods, he concluded that dextrose is the first apparent sugar to appear. The appearance of levulose, and later of sucrose in the leaf veins, led him to believe that sucrose is the final sugar and is transported as such through the stem. Pellet, in a discussion with Vivien (*15, p. 173*), concludes that sucrose, dextrose, and levulose are formed simultaneously in the leaf and descend to the root as such.

The variations of sugars at different stages of maturity of the plant have been studied by different investigators. Colin (*4*) found that, in the early stages of growth of the sugar beet root, the reducing sugars may form as much as 20 per cent of the total sugars. As the root matures the proportion of reducing sugars to sucrose decreases, but the former are never entirely absent and are always most abundant at the growing tip. Davis (*6*) and his associates, work-

ing with the mangel, found that during the early stages of growth, when leaf formation is the main function, sucrose exceeds the hexoses in the leaves, but that later in the season, when sugar is being stored in the root, the hexoses predominate. They further noted

FIG. 8—Effect of parent stem on offspring. Left, stunted secondary growth with parent stem dead; center, upper, first generation rootstock attached to seed. This small, cylindrical type has only the seed to support its growth. Center, lower, two stunted offspring with no parent stem, it having been removed several months previous; and right, two vigorous offspring with good parent stem

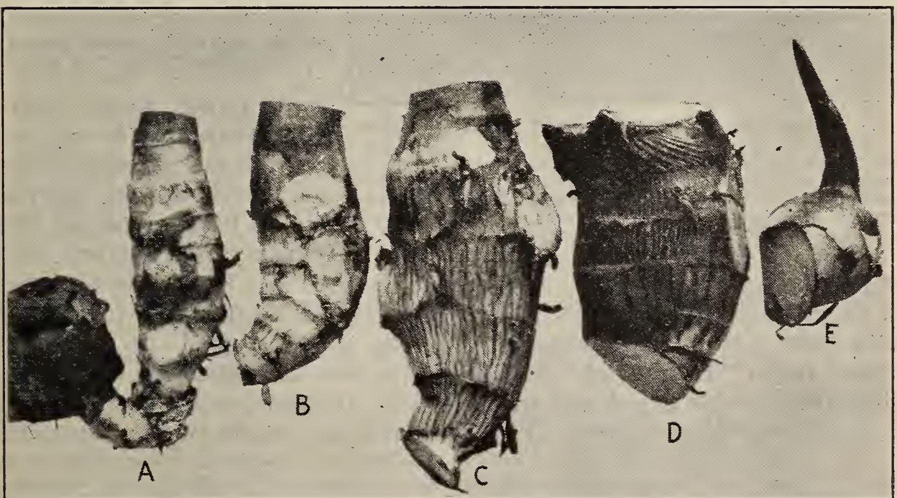

FIG. 9.—The progressive changes in shape of rootstocks in the hill. A, original seed with first generation cylindrical rootstock attached; B, second generation, small, intermediate type; C, third generation, large, intermediate type; D, fourth generation, surface type; E, fifth generation, surface type; spike rootstock, partly developed

that the hexoses always are in excess of sucrose in the midribs and stems, and that the ratio of hexoses to sucrose increases rapidly in passing down the stem to the root. The hexoses vary greatly during the day and night and throughout the season, whereas the sucrose

remains practically constant. Although the mangel is not a starch-storing crop, starch is found in its leaves in the early stages of growth, and disappears permanently as soon as the root begins to develop.

In studying the variations in the carbohydrates of the leaves of the potato plant during a 24-hour period, Davis and Sawyer (*5*) observed that the sucrose in the leaves reaches its maximum at 2 p. m. Subsequent decrease is accompanied by an increase in the hexoses and the appearance of soluble starch in the leaves, indicating a change of sucrose into invert sugar and subsequent condensation into starch. The starch decreases during the afternoon and early part of the night, reaching a very low value between midnight and 2 a. m., apparently being converted into hexoses.

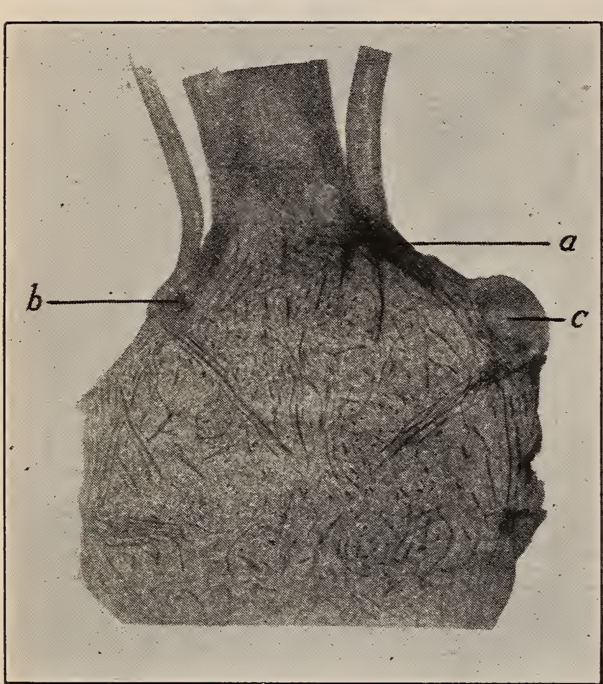

FIG. 10.—Method of development of buds. Longitudinal section of a portion of a young surface rootstock. The bud develops at the base of the scale. The vascular bundles supporting its growth are to be seen extending far into the interior. Under normal conditions, B and C develop as vigorous primary growth, whereas A either fails to develop or appears as secondary growth at a later period, when it is known as a "top bud"

Miller (*11*), working with the leaves of corn and sorghum, noted that the nonreducing sugars increase during the day and decrease during the night; whereas, the variations in the reducing sugars are comparatively small and very irregular. The insoluble carbohydrates, reaching a maximum later in the day than the sugars, decrease rapidly after midnight.

VARIATIONS IN DIFFERENT PARTS OF THE PLANT

Analyses were made to determine the nature of the carbohydrates formed by photosynthesis in the leaf of the canna plant, and any change taking place during transposition through the stem to their final deposition as starch in the rootstock. The sap was extracted from a fresh part of each sample[6] and the sugars were determined as outlined under "Laboratory Methods," p. 6. The remainder of some of the samples was dried and ground, and the percentage of moisture and starch was determined. Table 6 gives the results of the analyses.

[6] These and subsequent samples for sugar determination were dug between 9 and 12 a. m. While the time of sampling has a decided effect on the sugar content of the leaves of a plant, the stems and roots are much less subject to daily variation. The results obtained would hardly be materially affected by the time of taking the sample in the case of the stem and rootstock.

TABLE 6.—*Composition of different parts of the canna plant*

Sample No.	Source and parts of the individual plant	Moisture	Dry matter	Starch: Green weight	Starch: Dry weight	Solids other than starch	Sucrose in sap	Hexoses in sap	Total sugars in sap
		Per cent	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*
	HILL NO. 1 (11 MONTHS OLD), STATION FIELD 26C								
	Group 1:								
1	Stem	91.96	8.04				0.45	0.82	1.27
2	Rootstock (apex) [1]	68.25	31.75	23.11	72.78	8.64	1.57	.25	1.82
3	Rootstock (base) [1]	66.67	33.33	26.18	78.54	7.15	1.47	.03	1.50
	Group 2:								
4	Leaves	77.20	22.80				1.54	.11	1.65
5	Midribs and sheaths	89.20	10.80				.94	1.50	2.44
6	Stem	90.46	9.54				1.18	2.55	3.73
7	Rootstock (apex)	72.62	27.38	20.29	74.09	7.09	2.53	.77	3.30
8	Rootstock (base)	66.91	33.09	26.53	80.19	6.56	1.87	.09	1.96
	Young Group 3a:								
9	Midribs and sheaths	92.91	7.09				.32	.29	.61
10	Rootstock (apex)	84.88	15.12	9.90	65.45	5.22	1.09	.43	1.52
11	Rootstock (base)	78.75	21.25	13.93	65.54	7.32	1.15	.29	1.44
	HILL NO. 2 (7 MONTHS OLD), STATION FIELD 26B								
	Group 2:								
12	Leaves						1.30	0.25	1.55
13	Midribs and sheaths						.87	1.18	2.05
14	Stem						1.68	2.48	4.16
	Group 3a:								
15	Leaves						1.49	.18	1.67
16	Midribs and sheaths						.63	1.33	1.96
	Young Group 3a:								
17	Leaves						1.00	.04	1.04
18	Midribs and sheaths						.75	.39	1.14
	HILL NO. 3 (3 MONTHS OLD), STATION FIELD 32C								
	Old Group 3:								
19	Leaves	82.29	17.71	0.248	1.40	17.46	2.61	0.04	2.65
20	Midribs and sheaths	92.00	8.00	.035	.44	7.96	.78	.59	1.37
21	Stem	94.72	5.28	.030	.56	5.25	.25	.82	1.07
22	Rootstock	89.27	10.73	4.900	45.70	5.83	2.13	.57	2.70
	HILL NO. 4 (12 MONTHS OLD), WAIMEA FIELD W-S								
	Old Group 2:								
23	Rootstock (apex)						0.70	0.21	0.91
24	Rootstock (base)						.60	.04	.64
	Group 3a: [2]								
25	Rootstock (apex)						1.20	1.02	2.22
26	Rootstock (base)						1.30	.76	2.06
	Group 3b: [2]								
27	Rootstock (apex)						.75	1.55	2.30
28	Rootstock (base)						1.20	1.22	2.42

[1] "Apex" denotes the apical and "base" the basal half of the rootstock.
[2] Groups 3a and 3b samples of hill No. 4 were exceptionally vigorous and large.

Samples Nos. 4 to 8, inclusive, Group 2, hill 1, showed the complete series of changes taking place in a mature plant from the formation of sugars in the leaves by photosynthesis to the storage of sugars as starch in the basal part of the rootstock. Starting with the leaves (fig. 15), the hexoses increased rapidly in progressing down the sheaths, midribs, and through the stem proper, then decreased in the apical part of the rootstock, finally falling practically to the same level in the basal part as in the leaves. The sucrose started

with a relatively high percentage in the leaves, dropped in the midribs and sheaths, rose in the apical part of the rootstock, and dropped again in the basal part. The total sugars had the same curve as the hexoses, although the variation was relatively small.

The leaves and sheaths of Group 1, hill 1, samples Nos. 1, 2, and 3, were dead and the stem was shrivelling at the apex. The sugars in the remaining part of the stem showed a ratio similar to that of the sugars of the stem of Group 2, although the quantity of each was considerably less. The total sugars within the rootstock were distinctly less than in Group 2, particularly in the apical part.

The stems of young Group 3a, hill 1 (samples Nos. 9, 10, and 11) had not developed to any appreciable extent and were immature, and the leaves were appearing at the top. The midribs and sheaths showed a very striking decrease in sugars as compared with Group 2,

FIG. 11.—Secondary growth in a field of stunted plants 12 months old. Severe drought practically destroyed stem growth. The developing secondary growth is very stunted with small undersized rootstocks

particularly in the hexoses, which fell below the sucrose; and the relationship between the apical and basal parts of the rootstock was different from that of the preceding groups. Sucrose increased from apex to base, whereas in Groups 1 and 2 it decreased. In the basal portions of these latter groups the hexoses were extremely low, but in Group 3a they were present in appreciable and only slightly less quantities than in the apical part.

In hill 2 (samples Nos. 12 to 18, inclusive) the stems showed a striking similarity to those of hill 1. Sample No. 14, a Group 2 stem, showed the same increase in sucrose over midribs and sheaths as was noted in sample No. 6, hill 1. Likewise, the midribs and sheaths of young Group 3a showed a low concentration of hexoses similar to that of sample No. 9, hill 1. The proportions of sugars of the leaves of Group 2, hill 2, old Group 3a, hill 3, and Group 2, hill 1, were very

similar. Notwithstanding a distinct falling off in the quantity of both sugars in the leaves of young Group 3a, hill 2, the ratio was practically the same.

Samples Nos. 19 to 22, inclusive, old Group 3a, hill 3, showed the same general composition and changes of sugars in transposition noted in hills 1 and 2. A number of significant differences occurred, however. The leaves were distinctly higher in sucrose but very low in hexose content. Sucrose continued to decrease in passing from midribs and sheaths into stem whereas in the mature plants of hills 1 and 2 it always increased. The total sugars decreased sharply in passing from the leaves downward into the stem, whereas in hills 1 and 2 the reverse was true. The rootstock was outstandingly higher in sucrose as compared with Group 3a, hill 1. The stem of

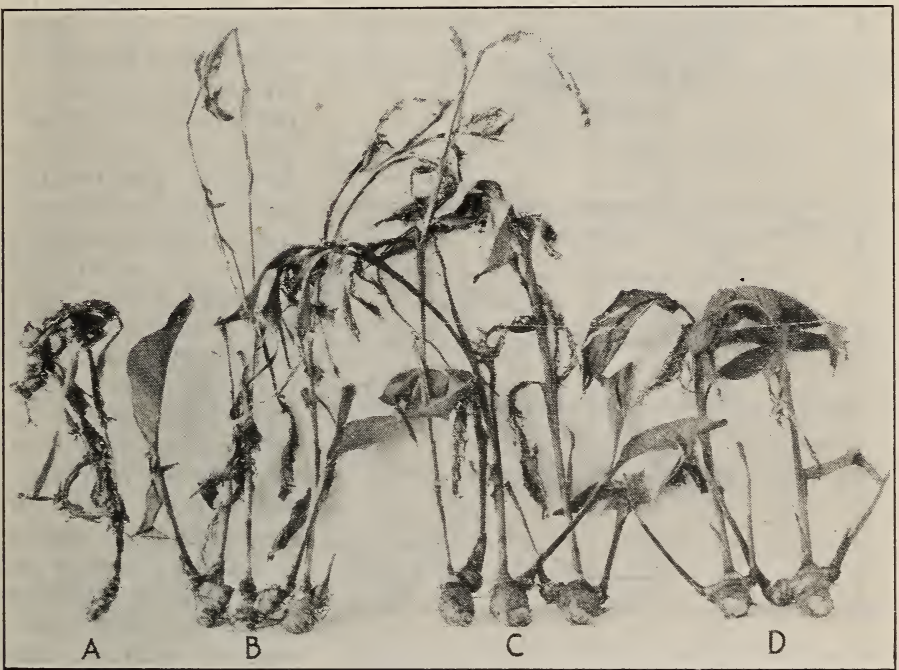

FIG. 12.—The result of stunting. A, B, C, and D represent the first, second, third, and fourth generations, respectively. Note the profusion of small, stunted "secondary" growth on all generations except the first

the plant contained starch in appreciable quantities throughout, whereas the stems of the other hills, even in young Group 3a, hill 2, contained only traces of starch.

Samples Nos. 23 to 28, inclusive, hill 4, from Waimea, showed the extreme variations occurring within a single hill. Samples Nos. 23 and 24, the apical and basal parts of a rootstock the stem of which was practically dead, were very low in sucrose and hexose content. Both sugars increased greatly, particularly the hexose, in samples Nos. 25 and 26, a vigorous Group 3a rootstock. This increase continued, the hexoses exceeding the sucrose, in samples Nos. 27 and 28, a vigorous Group 3b rootstock. In Groups 2 and 3a, the total sugars decreased in passing from the apical to the basal part, whereas, in Group 3b, they slightly increased. The basal part of the Group 2

rootstock was lower than the apical part in sucrose content, whereas the reverse was true in Groups 3a and 3b. The hexose content of the apical parts of all three groups was higher than the basal. Although the actual percentage of each was radically different from those of the rootstocks of hill 1, grown at the station, the above-mentioned relations held true in the latter.

Some very distinct differences were apparent in the variations of the components other than sugars in hills 1 and 3. In Groups 1 and 2, hill 1, the average total dry matter and starch content of the rootstock were practically the same, whereas in Group 3a they showed a sharp decrease. The percentage of starch in the dry matter also decreased. In all three groups the basal part of the rootstock contained the larger quantity of starch. In Groups 1 and 2 the proportion of starch in the total dry matter was distinctly greater in the basal part, whereas in the Group 3a rootstock the percentage, although lower than in the former groups, was practically the same in both parts. Hill 3 was noticeably very low in average total dry matter and starch content of both rootstock and dry matter.

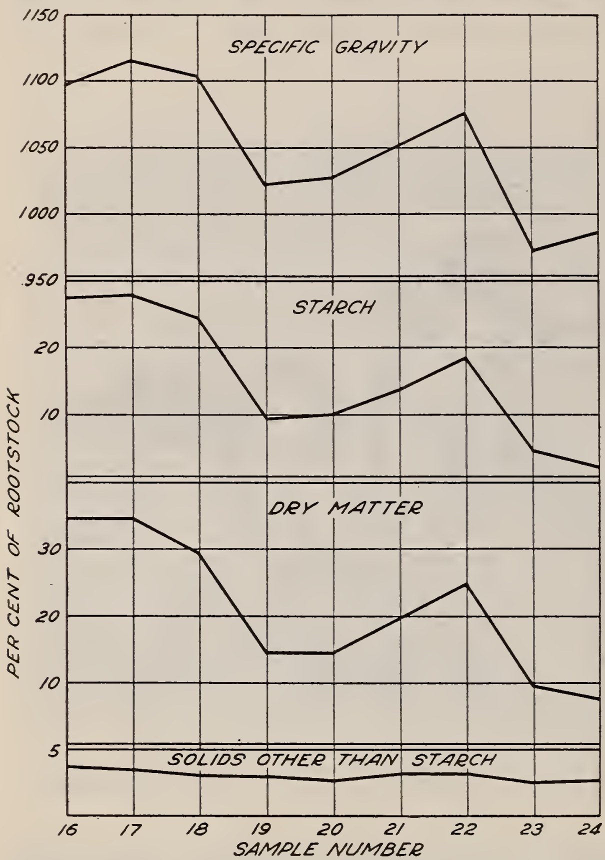

FIG. 13.—Relation of specific gravity to starch, dry matter, and solids other than starch in canna rootstocks

Table 6 presents some very definite evidences as to the nature of the sugars of the canna plant and the changes occurring in their translocation from the leaves to the rootstock. The chief sugar of the leaves is sucrose with a small but ever present quantity of the hexoses. The hexose-sucrose ratio remains consistently low regardless of the age of the hill and the stage of maturity of the plant. This is notable in considering the data of Davis and Sawyer (*5*), who found, in case of the potato plant, that although sucrose predominates in early growth, the hexoses exceed sucrose in later growth when translocation rather than growth is the principal function of the leaves; whereas, the results of Lutman (*9*) and Miller (*11*) show very little correlation between the reducing and nonreducing sugars of the leaves.

The method of extracting sap by means of a meat grinder and screw press is not well adapted to the leaves of the canna plant because the sap is very viscous. Moreover, it was necessary to use all the leaves from a single stem to obtain enough sap for a determination. Possibly a more complete extraction, using greater pressure as is suggested by Knudson and Ginsburg (*8*), might affect the results somewhat. The constancy of the data would seem sufficient to substantiate the essential correctness of the conclusions drawn, however.

The very rapid rise of the hexoses and the decrease of sucrose in the midribs and sheaths and the continuation of these changes in the

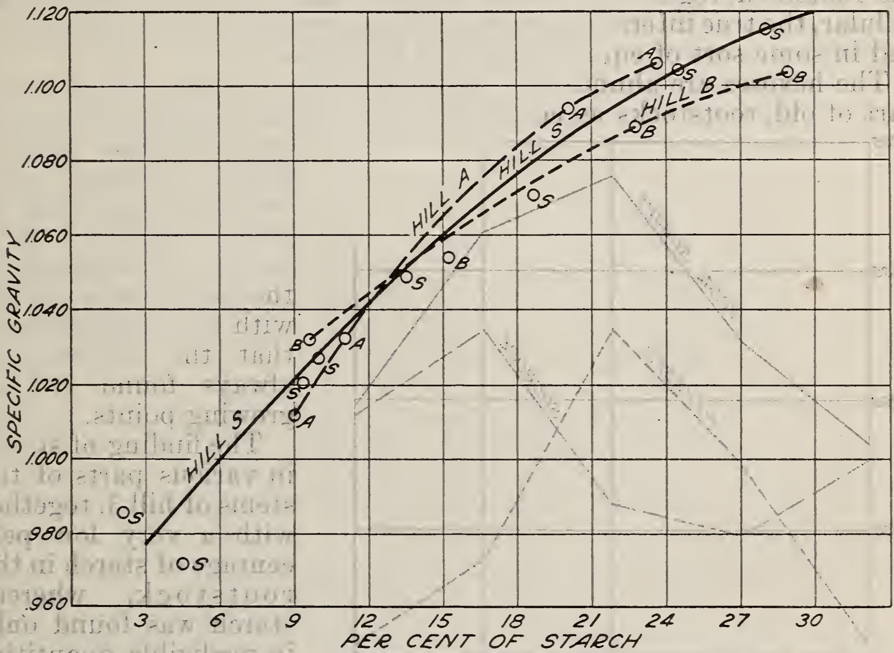

FIG. 14.—Relation of specific gravity to percentage of starch in canna rootstocks

stems is strong evidence in favor of the hexoses as the sugars concerned in translocation. This is in accord with the findings of Davis and Sawyer.

In the stems of Group 2, hills 1 and 2, the hexoses still greatly exceeded the sucrose, but the latter showed an increase in concentration over that of the sheaths and midribs. This probably resulted from the functioning of the stem as a temporary storage organ. In the stem of the younger plant, hill 3, no such phenomenon occurred, the sucrose decreasing to a very low percentage. Evidently no storage of sugars occurred; in fact, the total sugars very markedly decreased. In explanation it may be said that the stem was in process of rapid growth during which food material was not stored, but rather diverted toward the apex of the stem.

In all instances the sucrose suddenly increased upon reaching the rootstock and the hexoses correspondingly decreased. This fact further confirms the conclusion that inversion of sucrose to hexoses is primarily for the purpose of translocation. The hexoses again decreased rapidly toward the basal part of the rootstock. They were seldom entirely wanting, and were found in smallest quantity in regions where translocation was at a minimum and, likewise, in greatest quantity where it was at a maximum, confirming the opinion that they practically alone effect translocation. In the basal part of the rootstock sucrose decreases, of course, as the result of its condensation into starch. The occurrence of sucrose in very appreciable quantities in the rootstock, regardless of age, suggests that it is essentially intracellular, the true intermediate product between the hexoses and starch, and in some sort of equilibrium with the latter.

The hexoses are abundantly present in both young, and the apical part of old, rootstocks as compared with the basal part of old rootstocks, a fact offering further evidence that reducing sugars are always associated with growth. This bears out the observation of Colin with the sugar beet (*4*) that the hexoses are always found at the growing points.

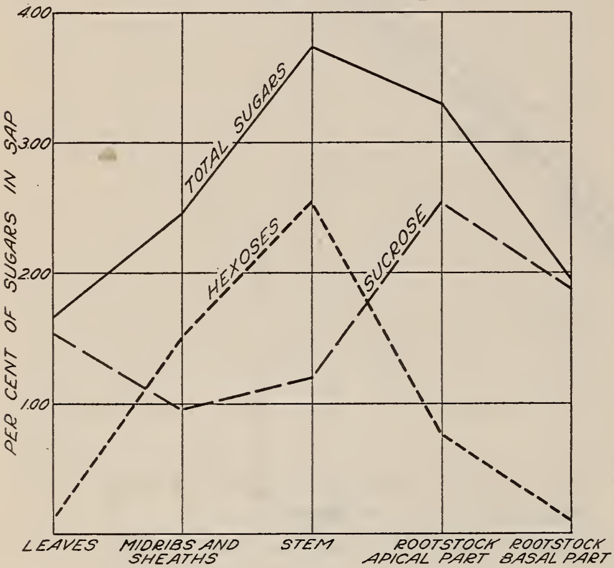

FIG. 15.—Sugars in different parts of the plant

The finding of starch in various parts of the stems of hill 3, together with a very low percentage of starch in the rootstock, whereas starch was found only in negligible quantities in the stem of the older hills, is in accord with the observations of Davis, Daish, and Sawyer (*6*), who showed that starch forms in the leaves of the mangel plant only during its very early growth. In explanation of this phenomenon they advanced the hypothesis that starch was stored in the leaves because the root of the mangel had not yet developed, but that as soon as sugar began to be deposited in it the starch disappeared from the leaves and did not reappear.

VARIATIONS IN THE SAME HILL

A number of hills of different ages were classified and genealogized in the usual manner to correlate the variations taking place in the composition of the plants with the growth of the hill in the field. Representative plants from each generation were selected for determination of their sugars and some other constituents. Table 7 gives the results of the analysis of the stem[7] and the rootstock.

[7] The term "stem" is here used to include the sheaths and the stem proper, the leaves and the midribs being discarded. To obviate the possibility of includiug a part of the stem with the rootstock, and vice versa, a section of the apical part of the rootstock and the lower part of the stem, about 2 inches, was discarded.

TABLE 7.—*Composition of different plants within a single hill*

Laboratory No.	Generation	Stage of maturity	Moisture: Rootstock	Moisture: Stem	Dry matter: Rootstock	Dry matter: Stem	Starch, Green weight: Rootstock	Starch, Green weight: Stem	Starch, Dry weight: Rootstock	Starch, Dry weight: Stem	Solids other than starch in rootstock	Sucrose in sap: Rootstock	Sucrose in sap: Stem	Hexoses in sap: Rootstock	Hexoses in sap: Stem	Total sugars in sap: Rootstock	Total sugars in sap: Stem
			Per cent	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*
		HILL NO. 1 (11 MONTHS OLD), STATION FIELD 26C															
1	First	Old Group 1	73.37		26.63		18.41		69.14		8.22	1.69		0.36		2.05	
2	Second	Medium Group 1	66.69		33.31		25.83		77.53		7.48	1.61		.19		1.80	
3	Third	Old Group 2	65.27	90.83	34.73	9.17	27.34		78.73		7.39	2.23	0.50	.15	2.01	2.38	2.51
4	Fourth	Young Group 2	67.82	90.88	32.18	9.12	25.15		78.15		7.03	1.50	.59	.20	1.73	1.70	2.32
5	Fifth	Medium Group 3a	74.18	93.20	25.82	6.80	19.65		76.09		6.17	1.23	.19	.21	.94	1.44	1.13
6		Group 3b (spike)	86.81		13.19		8.26		62.65		4.93	.94		.43		1.37	
		HILL NO. 2 (7 MONTHS OLD), STATION FIELD 26B															
7	First	Medium Group 2	67.92	90.50	32.08	9.50	22.95		71.54		9.13	2.15	0.63	0.15	1.57	2.30	2.20
8	Second	do	66.65	89.07	33.35	10.93	25.52		76.53		7.83	2.12	1.01	.18	1.93	2.30	2.94
9	Third	Young Group 2	68.60	89.48	31.40	10.52	23.88		76.05		7.52	1.71	1.00	.05	1.96	1.76	2.96
10	Fourth	Young Group 3a	80.44	93.58	19.56	6.42	14.38		73.50		5.18	1.37	.26	.44	.47	1.81	.73
11		Group 3b (spike)	80.18		19.82		13.98		70.54		5.84	.45		.06		.51	
		HILL NO. 3 (3 MONTHS OLD), STATION FIELD 32C															
12	First	Old Group 3a	88.90	93.32	11.10	6.68	4.36	0.037	39.33	0.55	6.74	2.33	0.32	0.45	0.58	2.78	0.90
13	Second	Medium Group 3a	89.64	93.77	10.36	6.23	3.75	.042	36.17	.68	6.61	1.93	.60	.69	.74	2.62	1.34
14	Third	Young Group 3a	92.36	93.83	7.64	6.17	1.74	.042	22.80	.68	5.90	1.24	.23	1.02	.31	2.26	.54
15		Group 3b (spike)	93.68		6.32		.65		10.24		5.67	.88		1.22		2.10	
		HILL NO. 4 (12 MONTHS OLD), WAIMEA FIELD W-S															
16	First	Old Group 2										1.98	0.51	0.60	3.03	2.58	3.54
17	Second	Medium Group 2										2.05	.48	.13	2.64	2.18	3.12
18	Third	Old Group 3a										1.55	.45	.21	2.48	1.76	2.93
19	Fourth	Medium Group 3a										1.55	.20	.44	.92	1.99	1.12
20	Fifth	Group 3b (spike)										.90		.74		1.64	

In hill 1, 11 months old, sample No. 3, the rootstock and stem of an old Group 2 plant, contained the maximum total sugar. (Fig. 16.) In this stage all new growth had ceased and the lower leaves of the plant had died, leaving only the apical leaves to function. Throughout subsequent generations the sugars decreased rapidly. In the stem of sample No. 5, Group 3a, which was still growing at the top, both sucrose and the hexoses sharply decreased. Sucrose remained low and constant in the stem throughout Group 2, whereas the hexoses were much higher. The reverse was true of the rootstock, the hexose content being very low and constant throughout Groups 1 and 2, with the sucrose content much higher and more variable. Sucrose decreased in sample No. 6, Group 3b, whereas the hexose content was double that of sample No. 5. A comparison of the total sugars in the rootstock and the stem shows that in the Group 2 stage the stem had a greater concentration than the rootstock, whereas in Group 3a the reverse was true, although in most cases the differences were small. Marked variations also occurred in the other constituents. The rootstock of sample No. 3 contained the maximum dry matter and starch content, both of green and dry weight, and also the maximum sugar content. These values were remarkably constant in samples Nos. 2, 3, and 4, Groups 1 and 2. Sample No. 5, a Group 3a rootstock, showed a decrease, and sample No. 6, a Group 3b rootstock, a very sharp decrease. The solids other than starch showed a similar decrease, except that the maximum value was in sample No. 1, the first generation rootstock.

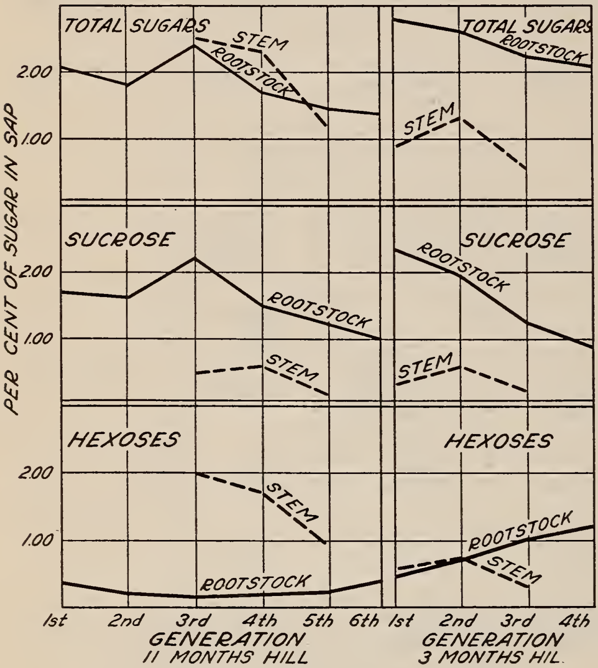

FIG. 16.—Sugars in different plants within the hill

A consideration of hill 2, 7 months old, shows that it closely resembles hill 1, in an adjacent field, except that the sucrose of the stems of Group 2 was higher. The decrease, rather than increase, of the hexoses in sample No. 11 indicates that the rootstock was old with meristem dead, instead of young and fresh as was supposed.

A number of striking differences, particularly with regard to the hexoses of the rootstocks, were observed between hill 3, 3 months old, and hill 1, grown in an adjacent field. Beginning with sample No. 12, the hexoses were about one-fifth that of the sucrose. The hexoses increased rapidly and the sucrose decreased until in sample No. 15 the former exceeded the latter. The hexoses of the stems were very low. The total sugars of the Groups 3a and 3b rootstocks were unusually high, owing largely to their high hexose content,

whereas, the total sugars of the stems were far lower than those of the rootstocks, owing primarily to their low hexose content. The very low percentages both of dry matter and of starch in the rootstock were in contrast to the hills previously mentioned. The solids other than starch had practically the same values as hills 1 and 2. The occurrence of starch in the stems has been previously noted in a similar hill. (Table 6, hill 3.)

Within the stem the hexoses seem to be an index of the amount of material being furnished to the rootstock for starch formation. The sucrose has a more constant value and shows less correlation with the growth of the hill. In the rootstock, however, the sucrose follows the variations of the hexoses of the stem rather closely and thus furnishes a possible index of the rate of storage of starch. The hexoses remain low and fairly constant in the mature rootstock, but increase in concentration in the youngest growth, and may be considered as an index of the rate of cell growth in the young rootstock.

It is a significant fact that the curve for the sucrose content of the rootstocks follows very closely that of the hexoses of the corresponding stems. Davis, Daish, and Sawyer (*6*) suggest that, in case of the sugar beet, a larger quantity of hexoses is found when the hexoses enter the root in excess of its "saccharifying power." The accumulation of sucrose in the edible canna rootstock might be due to its production in excess of its "amylogenic power," in which case the increase in the sucrose of the older groups might be attributed to a decrease in this power rather than to any increase in the rate of formation of starch. That starch formation was still taking place was shown by the increase in the percentage of starch over that of preceding groups. Evidently change from hexoses to sucrose was rapid, since in no instance did the hexose content of a Group 2 rootstock show any increase, regardless of the percentage of hexoses in the stem. The high concentration of the hexoses in the stems of Group 2 would seem to indicate that this is the stage in which the stem sends the largest quantity of sugars to the rootstock. Sample No. 3, old Group 2, had the highest sugar content notwithstanding the fact that it had only few green leaves at the top.

In Group 3a, comprising immature stems, the sugars decreased sharply. This indicates that much of the synthesized plant foods are carried to the growing portions of the stem instead of to the rootstock, and that the stem does not function at its maximum in supplying sugars to the rootstock until all new growth ceases.

The hexoses within the rootstock of Groups 1, 2, and 3a were uniformly low. The slight effect on the hexoses of the large variations in the stems as well as in the sucrose of the rootstocks in Groups 2 and 3a is surprising. Using the reducing sugars as a criterion, it is seen that, in Groups 2 and 3a, the sugars were carried down the stem at the maximum rate although active cell growth of the rootstock had largely ceased. This would seem to indicate that the process of starch formation within the rootstock continues long after active cell growth ceases. Reference to the starch content of the different groups tends to bear out this observation. In sample No. 6 cell growth was still taking place since the stem had not begun to develop and the apical growth was meristematic. The hexoses were comparatively high, whereas the starch content was very low. The stem

proper begins to ascend the stalk with the development of the first leaf, and cell growth at the apex of the rootstock probably then rapidly decreases. In sample No. 5, Group 3a, the hexoses decreased to the same level as in the mature rootstocks of Groups 1 and 2. The starch, on the other hand, increased rapidly, the increase continuing through the Group 2 stage.[8]

A consideration of hill 3 shows that the entire hill was actively growing, the first generation still being in the Group 3a stage. This explains the low concentration of sugars in the stem. The presence of such large quantities of sugars, particularly of the hexoses in the rootstock, is noteworthy. The very low starch content in the rootstocks and the occurrence of starch in the stems leads to the conclusion that the hill was still in the process of establishment and that the rootstock had not begun to function with sufficient rapidity to convert the sugars into starch as fast as they were formed.

Hill 4, 12 months old, from Waimea, showed variations in sugar very similar to those of hills 1 and 2 from the station. The hexoses of the stems were appreciably higher than in samples from the station. In view of the important part the mature stem apparently plays in the process of starch storage, it is significant that the hill had three generations of stems all of which functioned at nearly a maximum rate, whereas hill 1, which was of practically the same age, had only one generation.

The solids other than starch, representing the resultant of all the other variable constituents, can be interpreted only in a very general way. Except for the first generation rootstocks, which are essentially different in structure from the others in the hill, the proportions of the solids other than starch were relatively constant as compared with the corresponding percentages of starch. Sample No. 15, for example, had only 0.65 per cent of starch, but was less by only 1.81 per cent in solids other than starch than sample No. 2, which contained 25.83 per cent of starch. This appears to indicate that starch formation is the principal change taking place in the rootstock approaching maturity.

A comparison of the analyses with data previously published by the station (*3, p. 7*) shows similar variations, depending upon the stage of maturity and age of the hill. The nitrogen-free extract, as shown in the earlier literature, was appreciably higher than was the proportion of starch in these investigations, because, in the former instance, all other carbohydrates hydrolyzed by 1.25 per cent sulphuric acid were included, whereas in this paper only the true starch as determined by the diastase method is given.

VARIATIONS IN PLANTS FROM IRRIGATED AND UNIRRIGATED HILLS

Two adjacent plats at the station were planted with canna and irrigated until sprouting occurred. Irrigation was then discontinued on one plat, but continued every fifth day on the other. During the hot, dry summer which followed, the unirrigated plat produced a very stunted crop, whereas the plants on the irrigated plat made

[8]As previously stated (p. 2), the rootstock continues to increase in size, as was shown by actual measurements in the field and the occurrence of fresh cracks on the surface of rootstocks in old Groups 3a and 2. The phenomenon is noticeable particularly at Waimea.

vigorous growth. A representative hill from each plat was dug four and one-half months after planting for determination of sugar. Table 8 shows the difference in the sugars of hills from the two plats.

TABLE 8.—*Sugars in 4½-months-old canna plants grown on irrigated and unirrigated plats in station field 26B*

IRRIGATED PLAT

Sample No.	Generation	Stage of maturity	Sucrose in sap		Hexoses in sap		Total sugars in sap	
			Rootstock	Stem	Rootstock	Stem	Rootstock	Stem
			Per cent	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*
3	First	Young Group 2	2.91	0.51	0.20	0.94	3.11	1.45
4	Second	Old Group 3a	2.69	.47	.33	1.25	3.02	1.72
5	Third	Medium Group 3a	1.70	.29	.32	.46	2.02	.75
6	Fourth	Group 3b (spike)	1.44		.51		1.95	

UNIRRIGATED PLAT

Sample No.	Generation	Stage of maturity	Sucrose in sap Rootstock	Sucrose in sap Stem	Hexoses in sap Rootstock	Hexoses in sap Stem	Total sugars in sap Rootstock	Total sugars in sap Stem
1	First	Medium Group 3a	1.32	0.37	0.26	0.31	1.58	0.68
2	Second	Young Group 3a	1.26	.33	.09	.08	1.35	.41

The sucrose content of the rootstocks of the irrigated field was practically double that of the unirrigated, whereas the hexoses were not appreciably higher except for the youngest rootstock. Likewise, the hexoses of the stems from the irrigated field were much higher than those from the unirrigated and the percentage of sucrose was only slightly greater. In the irrigated field the hexoses of the stems greatly surpassed the sucrose, whereas the reverse was true of the unirrigated field. The results point strongly to the observations previously noted that the hexoses of the stem and the sucrose of the rootstock are measures of the rate of translocation of sugars and storage of starch.

EFFECT OF IRRIGATING PLANTS STUNTED BY DROUGHT

Low results were obtained in an analysis of plants from a hill of canna from station field 32C, which three months previously showed a high sugar content (see Table 7, hill 3), but which had subsequently been subjected to a hot, dry period. To determine whether the severely stunted field would resume growth, with the production of good-sized rootstocks, a part of the field was irrigated every fifth day for a period of two months, when the sugars were determined in plants from both irrigated and unirrigated parts. Field 26A, on which the plants had to a considerable extent matured before the drought set in (Table 7, hill 2), was also included in the study. Table 9 gives the condensed results of analyses made from time to time, the average composition of the entire hill being given in each instance. It further gives, for the sake of comparison, the average sugar content of a hill taken from each field during the period of optimum growth.

TABLE 9.—*Effect of irrigation on the sugars of stunted plants*

HILL NO. 1, STATION FIELD 32C (PLANTED JAN. 10, 1925)

Laboratory No.	Date of harvest	Period of irrigation[1]	Sucrose in sap		Hexoses in sap		Total sugars in sap		Specific gravity of rootstock
			Rootstock	Stem	Rootstock	Stem	Rootstock	Stem	
	1925	*Days*	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*	
1	July 21	11	0.71	0.29	0.24	0.43	0 95	0.72	----------
[2] 2	---do----	0	.56	.31	.15	.18	.71	.49	1.041
3	July 28	18	.66	.46	.29	.33	.95	.79	1.053
4	Aug. 14	35	.59	.30	.24	.51	.83	.81	1.048
5	Sept. 8	60	.60	.29	.18	.39	.78	.68	1.036
[3] 6	Apr. 8	0	1.65	.36	.72	.54	2.37	.90	.991

HILL NO. 2, STATION FIELD 26A (PLANTED JAN. 10, 1925)

Laboratory No.	Date of harvest	Period of irrigation[1]	Sucrose in sap: Rootstock	Sucrose in sap: Stem	Hexoses in sap: Rootstock	Hexoses in sap: Stem	Total sugars in sap: Rootstock	Total sugars in sap: Stem	Specific gravity of rootstock
7	July 25	15	1.16	----------	0.08	----------	1.24	----------	1.063
[2] 8	---do----	0	1.16	----------	.13	----------	1.29	----------	----------
9	Aug. 26	32	2.47	.80	.21	0.91	2.68	1.71	1.068
[2] 10	---do----	0	1.30	.50	.19	1.20	1.49	1.70	1.070
11	Sept. 2	39	2.83	.58	.22	.79	3.05	1.37	1.066
[2] 12	---do----	0	1.03	.29	.15	.42	1.18	.71	----------
[3] 13	Dec. 4	0	1.29	.77	.21	1.85	1.50	2.62	1.071

[1] Irrigation begun July 10, 1925.
[2] Unirrigated portion of the field.
[3] Harvested at optimum growth.

The results show no increase in the sugars in the stunted plants of station field 32C after two months of constant irrigation. If the specific gravity of the rootstocks is used as an index of their starch content the starch is seen to have made no increase during the period, and the very low hexose content of the stem shows that only a very small quantity of sugar was being transported down the stem. In the unstunted plants of station field 26A, however, not only did the sucrose in the rootstocks increase, but the increase was more than double that of the hill during its optimum growing period notwithstanding the uniformly low hexose content of the stems and the almost constant specific gravity of the rootstocks. In both plats the new growth resulted in very small rootstocks and stunted stems, with even the second generation of renewed growth decidedly undersized.

The above apparent contradiction may be explained by the fact that the increase in sucrose in field 26A was in reality due to breaking down of the starch previously stored in the rootstock to nourish the buds developing on even the oldest rootstocks of the hill; whereas, in a vigorously growing hill the plant food is supplied by the parent stem. Failure of the sucrose to increase in case of field 32C may have been due to the fact that the hill selected for analysis was younger and the developing buds were on the youngest rootstocks the stems of which retained sufficient vitality to support a stunted new growth.

MATURE ROOTSTOCKS DEVOID OF STEMS

Many old rootstocks, the stems of which always remain as "spikes," form a part of every hill of canna. Failure to develop stems may be due to the death of the meristem, as evinced by blackening of the apex, or to the increased vigor of the offspring which take practically

all the nutrition from the parent stem. In addition to these old rootstocks, the hill contains the dormant rootstocks (Group 1 stage), the stems of which develop normally and then die back. The sugars of the mature "stemless" rootstocks are of interest because they represent the residual components of the rootstocks unaffected by incoming materials from the stem.

Table 10 shows the sugar content of a number of rootstocks which, for one reason or another, were devoid of stems.

TABLE 10.—*Sugars in sap of mature rootstocks devoid of stems*

Sample No.[1]	Description of rootstock	Sucrose in sap	Hexoses in sap	Total sugars in sap
		Per cent	*Per cent*	*Per cent*
1	Dormant; stem dead[2]	1.61	0.19	1.80
2	Nonirrigated; stem removed 5 months previous	.98	.22	1.20
3	Irrigated; stem removed 5 months previous	2.08	.22	2.30
4	Old, nonirrigated; spike dead[3]	1.09	.00	1.09
5	Old, irrigated; spike dead	2.65	.15	2.80
6	Old, grown at Waimea; spike dead	.51	.01	.52

[1] All samples, except No. 6, were grown at the station.
[2] Sample No. 2 of Table 4.
[3] Attached to sample No. 2.

The table shows that sample No. 1, the stem of which developed normally and subsequently died, had a comparatively high sucrose content. Sample No. 2, the stem of which had been removed while still green, and sample No. 4, the spike of which had died, showed decidedly lower concentrations of sucrose. The increase of sucrose content of samples Nos. 3 and 5 to more than double that of samples Nos. 2 and 4, respectively, further substantiates the theory previously advanced (p. 26) that increase in sucrose may be due to hydrolysis of the starch already formed in the rootstock, rather than to translocation from the stem, since the rootstocks were devoid of stems. Sample No. 4 was expected to represent the minimum concentration of both sucrose and the hexoses possible within a rootstock because it was supporting no growth and was attached to sample No. 2, which was without stem development. The hexose content was nil but the percentage of sucrose was not outstandingly low, sample No. 6, which was an old rootstock with dead spike, having less than half the quantity.

An abnormally low sucrose content in a mature rootstock has been frequently observed to accompany a low percentage of starch (determined by the specific gravity method), suggesting the possibility of the sucrose of the rootstock being in some sort of equilibrium with its starch, since a low sucrose content results from a cell structure "unsaturated" with starch, and a high sucrose content from a cell structure having a high starch content. This theory seems to be confirmed by Table 9, which shows that samples from the unirrigated section of station field 26A had a higher sucrose content than those from station field 32C, on which the crop became stunted before any of the rootstocks reached full maturity.

EFFECT OF STORAGE

Determinations of the sugar content of rootstocks kept for different lengths of time after digging in a covered jar at room temperature are given in Table 11.

TABLE 11.—*Effect of storage on sugar content of sap of canna rootstock*

Age of rootstock	Time stored	Sucrose in sap	Hexoses in sap	Total sugars in sap	Ratio of hexoses to sucrose
Months	*Days*	*Per cent*	*Per cent*	*Per cent*	
12	--------	1.16	0.13	1.29	1:0.112
12	8	2.62	.33	2.95	1:0.126
12	[1]15	3.73	.55	4.28	1:0.147
12	21	2.73	.37	3.10	1:0.135
6	30	3.06	.18	3.24	1:0.059

[1] Beginning to ferment.

The table shows that the sugars of the stored rootstock increased continuously for about two weeks, when fermentation began. The hexose-sucrose ratio also increased. The rootstock which had been stored for 21 days was decidedly spongy and showed signs of fermentation. In this case both forms of sugar decreased but the hexose-sucrose ratio was nearly the same as in the rootstock which had been stored for eight days. That this did not hold for all the rootstocks is shown by the different ratio obtained in rootstocks from a 6-months-old hill which had given no appreciable sign of fermentation after it had been stored for 30 days. Unfortunately, the original sugar content of this sample was not determined.

The results indicate the rapidity of the changes taking place in the canna rootstock after it is dug. Such changes would seem to be of considerable significance in extracting the starch for commercial uses. Although the rootstock can be left in the hill for a number of months after maturity without deteriorating, its value for starch manufacture is greatly impaired by a few days' exposure after digging. The starch granules remain unaffected for some time, but purification and sedimentation are rendered difficult by the large amount of slimy precipitate which settles out even before the rootstock shows sign of fermentation or decay.

DORMANT AND ACTIVELY GROWING PLANTS

At Waimea a field of canna developed neither new stems nor rootstocks during a period of about three months. However, the stems already developed were protected from injury by continuous mists and by windbreaks. Toward the end of the dormant period a hill was dug for sugar determination. Two months later the field showed vigorous development of both new stems and rootstocks. Another hill was then dug from the same section as the first. Table 12 compares the sugar content of the two hills.

TABLE 12.—*Differences in the sugars in sap of dormant and actively growing plants*

(DORMANT) HILL NO. 1, 13 MONTHS OLD, WAIMEA FIELD A

Laboratory sample No.	Generation	Stage of maturity	Sucrose in sap		Hexoses in sap		Total sugars in sap	
			Rootstock	Stem	Rootstock	Stem	Rootstock	Stem
			Per cent	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*
1	First	Old Group 2	0.96	0.64	0.18	1.25	1.14	1.89
2	Second	Medium Group 2	.76	.58	.13	1.53	.89	2.11
3	Third	do	.79	.91	.05	1.70	.84	2.61
4	Fourth	Young Group 2	.82	.41	.24	2.18	1.06	2.59
5	do	Old; spike, dead	.51		.01		.52	
6	Fifth	Old Group 3a	.99	.35	.24	.87	1.23	1.22
		(ACTIVELY GROWING) HILL NO. 2, 15 MONTHS OLD, WAIMEA FIELD A						
7	Third	Medium Group 2	1.82	1.34	0.19	3.41	2.01	4.75
8	Fourth	Old Group 3a	.90	1.01	.09	2.48	.99	3.49
9	Fifth	do	1.03	.90	.14	2.31	1.17	3.21
10	Sixth	Young Group 3a	1.51	.26	.33	.83	1.84	1.09
11	Seventh	Group 3b (spike)	1.46		.64		2.10	

[1] Sample No. 7, third generation, hill 2, is comparable with sample No. 3, hill 1, since the first and second generations were not included in the analysis of hill 2.

The dormant canna (fig. 17) was conspicuous by the abnormally low sucrose content of its rootstocks. The hexoses of the stems were also considerably below those previously noted in canna grown at Waimea. (Table 7, hill 4.)

Both the hexoses and the sucrose were greater in old stems of the vigorously growing canna than in the dormant canna. Although in sample No. 7, third generation of the actively growing canna, the sucrose increased, in samples 8 and 9 of the fourth and fifth generations, respectively, it showed little increase over samples Nos. 4 and 6 of the corresponding generations of the dormant canna, notwithstanding the increase in sugars in the stems. However, the sugars in the rootstocks of the sixth and seventh generations of the actively growing canna, representing the new growth, were rather normal for Groups 3a and 3b stages of development.

These results confirm observations previously made (p. 26) that usually when sugars within a rootstock decrease to a low level for a prolonged period they do not again increase unless hydrolysis of starch occurs. Whether this indicates a loss in amylogenic power in such rootstocks is not certain. That this is sometimes the case would seem to be indicated by the very low starch content frequently observed in rootstocks in the dormant stage, Group 1. The recovery of the Waimea field from a dormant period with subsequent production of good-sized rootstocks in its new growth, in striking contrast with the severe stunting of the new growth at the station, may be attributed to the unaffected condition of the stem growth, which continued to function, whereas at the station it was practically destroyed.

CRYOSCOPIC MEASUREMENTS AND OSMOTIC PRESSURE OF THE PLANT JUICES

The method of measuring osmotic pressure of expressed saps based on freezing-point depression has been extensively used in studying the growth of plants, their adaptability to different environments,

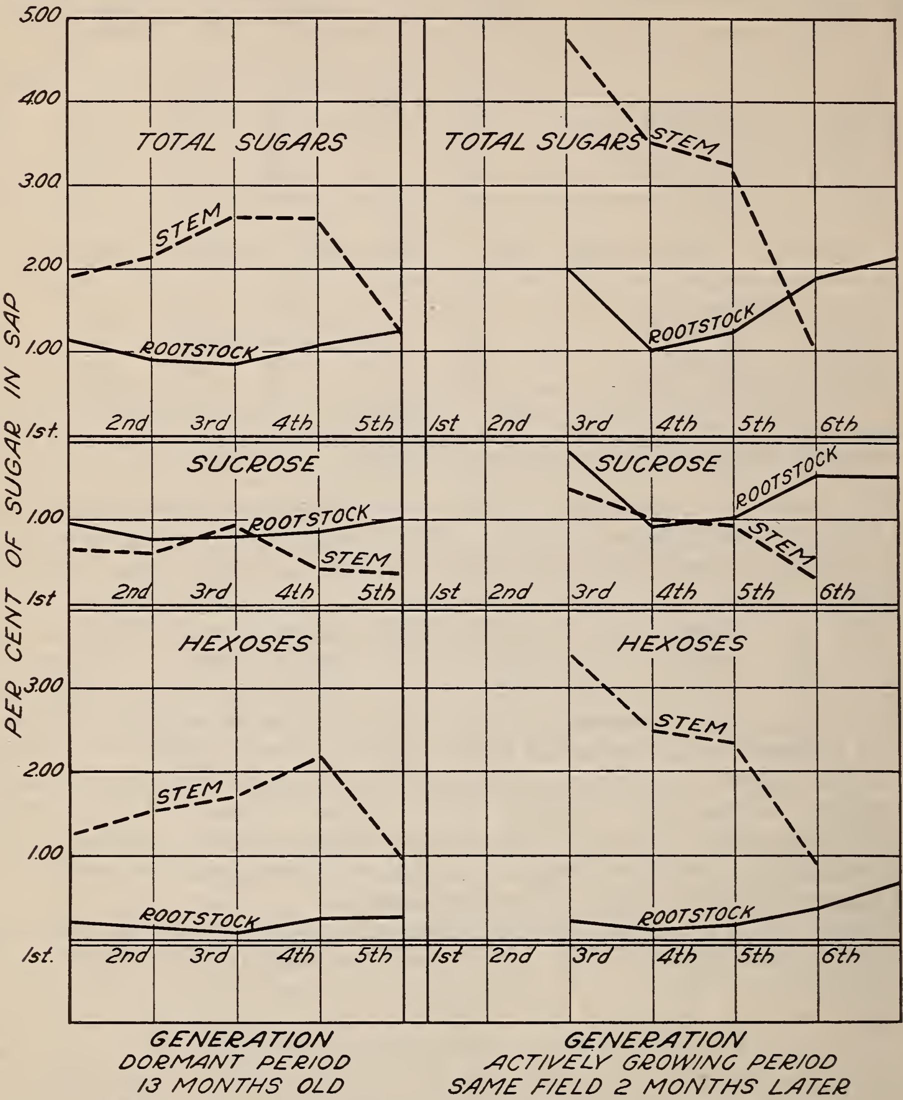

FIG. 17.—Sugars in a field of canna during a dormant and an actively growing period

and the movement of plant fluids in different parts of the organism. To learn whether results obtained by this method would substantiate the conclusions drawn from the preceding study of the sugars in the plant sap, determinations were made of the osmotic pressures of the tissue fluids from the rootstocks, stems, and leaves of canna plants, using cryoscopic measurements, as outlined by Harris and Gortner

(7).[9] Determinations were made in duplicate in the rootstock and stem samples. The results are given in Table 13.

TABLE 13.—*Osmotic pressure and freezing-point depression of sap from the canna plant*

Stage of maturity	Rootstocks		Stems		Leaves	
	Depression of the freezing point	Osmotic pressure	Depression of the freezing point	Osmotic pressure	Depression of the freezing point	Osmotic pressure
	° C.	*Atmosphere*	*° C.*	*Atmosphere*	*° C.*	*Atmosphere*
Group 1	0.789	9.502				
	.797	9.599				
Average	.793	9.551				
Group 2	.810	9.755	0.763	9.190		
	.833	10.933	.753	9.069		
Average	.822	10.344	.758	9.129		
Group 3a	.726	8.745	.804	9.683		
	.785	9.454	.821	9.887		
Average	.756	9.099	.813	9.785	0.683	8.227
Group 3b	.772	9.298	.614	7.397		
	.781	9.406	.694	8.360		
Average	.777	9.352	.654	7.879	.592	7.132

The results given in Table 13 are not sufficient to warrant definite conclusions. However, the close agreement of the results with the variations in the sugar content of similar samples is worthy of note. The osmotic pressure reached its maximum in the rootstocks of Group 2 and diminished through Groups 3a and 3b, which also was true of the sucrose. Since usually the hexoses in the rootstocks were negligible, the total sugars showed the same variation. The osmotic pressure in the stem reached its maximum in Group 3a, whereas the sugars were greatest in Group 2. However, the rise and subsequent decrease of the hexoses and total sugars in progressing from Group 1 to Group 3b were apparent in the osmotic pressure. Moreover, the differences in the depression of the freezing points of the rootstocks, expressed in terms of sucrose, were practically the same as the differences in the total sugars of the rootstocks given in Table 7. The same was true of the hexoses of the stems. The difference in the average depression of the freezing point of the rootstock of Groups 2 and 3b, for example, was 0.45° C., which is equivalent to 0.83 per cent sucrose, whereas the difference in the total sugars of Groups 2 and 3b (samples Nos. 3 and 6, Table 7), was 1.01 per cent. Although exact figures have no significance in this connection, since the freezing point and the sugar determinations were made on different samples representing the same group of rootstocks, the comparisons are worthy

[9] The determinations were made at the station in 1924 by Charles C. Crane, under the direction of J. A. Harris, department of botany, University of Minnesota. Doctors Harris and R. A. Gortner, also of the University of Minnesota, used the chemical laboratory facilities of the station in obtaining cryoscopic and conductivity data on Hawaiian plants, in exchange for which courtesy they collected the canna samples and turned over to the station the data accumulated.

of consideration. The leaves had the lowest osmotic pressure of any of the different parts of the plant. Reference to Table 7 shows that this was also true of the total sugars.

DISCUSSION OF RESULTS

The results obtained in this investigation lead to a number of practical conclusions. That the stem of the canna plant functions strongly in influencing the size of the offspring was confirmed by these results, which point, moreover, to the importance of the mature stem in storing starch within its own rootstock. The starch content of the rootstock is lowered when normal dying back of the leaves of the mature stem is unduly hastened by unfavorable weather. The fact that the immature stem fails to contribute much plant food to the rootstock and the mature stem continues to function at its maximum until it is well along in the mature stage emphasizes the importance of prolonging the life of the latter group.

A comparison of stem growth at the station and in Waimea shows that at the station the stem maintains its maximum rate of sugar production for a short time only, whereas in Waimea it persists for a much longer period. Although the loose, loamy soil of Waimea is probably better adapted to a root crop than are the heavy soils of the station, the climate, which prolongs the life of the mature stem, is especially favorable to the crop. The use of such artificial aids as windbreaks to protect the stem from damage would seem to be warranted.

The significance of the stem in maintaining the new growth of the hill is also brought out. Attempts at the station to renew growth on fields where the stems had been severely checked by drought resulted in a small crop of extremely stunted character, whereas at Waimea, where the stem growth had persisted through a long, dormant period, the new growth produced fairly good-sized rootstocks.

Usually, the starch content of a rootstock of the immature stage is low and steadily increases until the rootstock is well along in the mature stage. This fact would seem to warrant carefully surveying the field before harvesting, which is ordinarily done at a definite age, say, 15 months. If a large part of the growth is in the immature stage, the crop should be allowed to grow for an additional period so as to increase the starch content of the young rootstocks. On the other hand, when a dormant period prevents new rootstocks from forming at, say, 14 months, nearly all the rootstocks will be in the mature stage, and the crop may as well be harvested at once because the new rootstocks will not attain any appreciable starch content for several additional months. Inability of the crop to make renewed growth with the production of rootstocks of fairly good size after the top growth has been destroyed would seem to be sufficient reason for harvesting, regardless of the age of the crop. Since, under normal conditions, a field of canna is irregularly but continuously growing, it can never be definitely stated when the entire hill is mature, and a criterion other than age should be used to determine the best time to harvest.

Not only should the classification of the growth of a field be determined before harvesting but the approximate starch content of the

crop also should be ascertained by its specific gravity. A simple method consists in weighing a lot, say, 50 pounds, of cleansed rootstocks in a wire basket, first in air and then immersed in kerosene. The starch content can then be ascertained by referring to Table 5, page 11.

SUMMARY

In this series of experiments, made to study continuously the growth of edible canna (*Canna edulis*) and the occurrence of sugars and their changes during translocation and ultimate storage as starch in the plant, it was found that sucrose is the chief sugar of the leaves; the hexoses are present in only very small quantities, and the hexose-sucrose ratio is always very low. This is true of leaves from an old mature plant as well as from a young, rapidly growing plant. The percentage of sucrose is lower in the midribs and the sheaths than in the leaves, whereas that of the hexoses is much higher. In the stem proper the hexoses are present greatly in excess of the sucrose.

The sucrose in the apical part of the rootstock, as compared with the stem, increases and the hexoses correspondingly decrease. In the basal part of the rootstock both sugars usually decrease, especially the hexoses, which reach a very low percentage. The hexoses are therefore thought to be the chief sugars of translocation and the starch in the rootstocks to be formed from sucrose rather than from the hexoses.

A study of plants of different stages of maturity shows that the quantity of hexoses is much less in the immature stems than in the mature stem, owing to the diversion of the flow in the former to the apical growth of the plant. This indicates the value of the mature stem in the synthesis of food material. The sucrose content of the rootstocks shows a similar variation to the hexoses of the stem, the immature rootstocks having a much lower percentage of sucrose than the mature ones.

Young and vigorously growing rootstocks are characterized by a high percentage of the hexoses which occasionally exceed the sucrose. In the more mature rootstocks the hexoses are consistently low in quantity indicating their association with the growing parts of the rootstock. The concentrations of the hexoses in the stem and of sucrose in the rootstock would seem to be good indicators of the rate of transposition of sugars and their ultimate storage as starch in the rootstock. The sucrose of the stem and the hexoses of the rootstock are usually more constant and do not seem to correlate so well with growth, as determined by observations.

The sucrose content of the rootstock, especially one that is immature, is lowered when normal growth is halted for a protracted period. A return of favorable growing conditions often fails to cause any increase in the sucrose content. This fact, together with the low starch content of the rootstock, indicates a loss of amylogenic power.

Copious irrigation of a field of stunted plants may result in an increase of sucrose in the rootstock, but the increase seems to be due to the process of "germination," or hydrolysis of the starch already stored in the rootstock to support secondary growth development.

In the mature rootstock the sucrose content varies greatly under different conditions. Indications are that it exists in some sort of equilibrium with the starch, since the sucrose content is low in rootstocks having a low starch content and high in those having a high starch content.

When stem growth has not been injured during the dormant period, new growth with rootstocks of fairly good size is produced, whereas stunted stems produce extremely stunted rootstocks.

The percentage of starch varies widely in canna rootstocks of different stages of maturity. Starting with a low content in the very young rootstock, the starch increases up to the dormant stage of the rootstock, the increase being accompanied by an increase in specific gravity. Graphs of the starch show that although the "growth curve" of different hills varies somewhat the correlation between specific gravity and the percentage of starch is general. A table has been constructed (Table 5, p. 11), which makes it possible to determine in the field or the factory the approximate percentage of starch in a rootstock from its specific gravity.

Determinations of osmotic pressures by cryoscopic measurements were made with a limited number of plants. The results confirm the general conclusions regarding growth drawn from the study of the carbohydrate metabolism of the plant.

LITERATURE CITED

(1) Association of Official Agricultural Chemists.
1920. OFFICIAL AND TENTATIVE METHODS OF ANALYSIS. AS COMPILED BY THE COMMITTEE ON REVISION OF METHODS. REVISED TO NOV. 1, 1919. 417 p., illus. Washington, D. C.

(2) Brown, H. T., and Morris, G. H.
1893. A CONTRIBUTION TO THE CHEMISTRY AND PHYSIOLOGY OF FOLIAGE LEAVES. Jour. Chem. Soc. [London] 63:604–677.

(3) Chung, H. L., and Ripperton, J. C.
1924. EDIBLE CANNA IN HAWAII. Hawaii Agr. Expt. Sta. Bul. 54, 16 p., illus.

(4) Colin, H.
1914. SUR LA SACCHAROGÉNIE DANS LA BETTERAVE. Compt. Rend. Acad. Sci. [Paris] 159:687–689.

(5) Davis, W. A., and Sawyer, G. C.
1916. STUDIES OF THE FORMATION AND TRANSLOCATION OF CARBOHYDRATES IN PLANTS. III. THE CARBOHYDRATES OF THE LEAF AND LEAF STALKS OF THE POTATO. THE MECHANISM OF THE DEGRADATION OF STARCH IN THE LEAF. Jour. Agr. Sci. [England] 7:352–384, illus.

(6) ——— Daish, A. J., and Sawyer, G. C.
1916. STUDIES OF THE FORMATION AND TRANSLOCATION OF CARBOHYDRATES IN PLANTS. I. THE CARBOHYDRATES OF THE MANGOLD LEAF. Jour. Agr. Sci. [England] 7:255–326, illus.

(7) Harris, J. A., and Gortner, R. A.
1914. NOTES ON THE CALCULATION OF THE OSMOTIC PRESSURE OF EXPRESSED VEGETABLE SAPS FROM THE DEPRESSION OF THE FREEZING POINT . . . Amer. Jour. Bot. 1:75–78.

(8) Knudson, L., and Ginsburg, S.
1921. SUGGESTIONS WITH RESPECT TO THE MEASUREMENT OF OSMOTIC PRESSURE. Amer. Jour. Bot. 8:164–170, illus.

(9) Lutman, B. F.
1919. OSMOTIC PRESSURES IN THE POTATO PLANT AT VARIOUS STAGES OF GROWTH. Amer. Jour. Bot. 6:181–202, illus.

(10) Meyer, A.
1885. UEBER DIE ASSIMILATIONSPRODUCTE DER LAUBBLÄTTER ANGIOSPERMER PFLANZEN. Bot. Ztg. 43: 417–423, 433–440, 449–457, 465–472, 481–491, 497–507, illus.

(11) MILLER E. C.
1924. DAILY VARIATION OF THE CARBOHYDRATES IN THE LEAVES OF CORN AND THE SORGHUMS. Jour. Agr. Research 27 : 785–808, illus.

(12) SACHS, J.
1862. UEBER DEN EINFLUSS DES LICHTES AUF DIE BILDUNG DES AMYLUMS IN DEN CHLOROPHYLLKÖRNERN. Bot. Ztg. 20 : 365–373.

(13) SCHIMPER, A. F. W.
1885. UEBER BILDUNG UND WANDERUNG DER KOHLEHYDRATE IN DEN LAUBBLÄTTERN. Bot. Ztg. 43 : 737–743, 753–763, 769–787.

(14) STRAKOSCH, S.
1907. EIN BEITRAG ZUR KENNTNIS DES KOHLENHYDRATSTOFFWECHSELS VON BETA VULGARIS (ZUCKERRÜBE). Sitzber, Akad. Wiss. Wien, Math. Naturw. Kl. 116 : 855–869.

(15) VIVIEN, A.
1913. LA BETTERAVE EN EUROPE ET LA SACCHAROGÉNIE (I). Bul. Assoc. Chim. Sucr. et Distill. 31 : 164–172. (Discussion by A. Vivien and H. Pellet, p. 172–176.)

(16) WILEY, H. W.
1914. PRINCIPLES AND PRACTICE OF AGRICULTURAL ANALYSIS. Ed. 2, rev. and enl., v. 3, illus. Easton, Pa., and London, England.

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